

Sigma Xi Undergraduate Research Grant Proposal

The Prevalence and Genetic basis of Antibiotic Resistant *Escherichia coli* among College-Aged Adults.

1. Summary:

This study aims to investigate the prevalence of antibiotic resistant *Escherichia coli* among college-aged adults in the upper Midwest for six commonly used antibiotics. These strains of *E.coli* are not considered to be pathogenic. In order to assess resistance, R plasmids (small circular DNA molecules where resistance genes reside) will be purified for further plasmid profile analysis. The hypothesis is such that resistance likely exists for at least one type of antibiotic.

2. Background

The increasing use of antibiotics in healthcare since their discoveries has placed significant selective pressure on the ecosystem of bacteria, allowing those with antibiotic-resistance genes to thrive and eventually dominate. These genes are known to be located on various DNA molecules, including the chromosome, plasmids, transposons, and integrons (Barbosa and Levy, 2000). Most resistance genes reside on a type of plasmid called the R plasmid. Furthermore, it has been increasingly more common to find clinical isolates containing R plasmids with multiple antibiotic-resistant genes. These plasmids can be transferred from resistant cells to sensitive cells by means of conjugation, further adding to their distribution among human and animal populations. Such persistence and proliferation of antibiotic resistance in bacteria has made treatments less efficient while increasing the costs required for more potent agents. Consequently, antibiotic resistance has been one of the most pressing concerns of modern medicine (Anderson, 1999; WHO, 2001).

The bacterium *Escherichia coli*, an inhabitant of the lower intestine, will be utilized as the model system to examine the prevalence of antibiotic resistance among members of a small community such as our campus. The antibacterial tested will be ampicillin, penicillin G, tetracycline, streptomycin, ciprofloxacin, and sulfamethoxazole

3. Methods and Materials

Bacterial strains

E.coli will be collected from rectal swab samples taken personally by volunteer Gustavus students (n=20-30). The volunteers will gently dab the rectum with a sterile cotton swab to collect the sample in a bathroom outside of the microbiology laboratory. An application has been submitted to the IRB, pending approval (IRB application attached). Each sample will be assigned a culture ID (MP-month/date-xxx) to protect the anonymity of the volunteers. A questionnaire will be given to volunteers to obtain relevant information that may assist with data interpretation and analysis. (Questionnaire attached)

Bacterial isolation and identification

E.coli colonies will be isolated on eosin methylene blue (EMB) agar, following inoculating of the agar with a rectal swab sample. EMB is a classic medium for isolation of *E.coli* from clinical samples. Identified *E.coli* colonies will then be transferred to a separate agar for inoculation.

Antibiotics sensitivity testing

Eighteen-hour-old broth cultures from *E.coli* isolates will be spread on a Mueller-Hinton agar plates for antibiotic sensitivity testing using the disc diffusion method. Discs containing antibiotics will be dispensed from the cartridges onto the agar surface. After the incubation

period, measurement data of growth inhibition zones will be compared with standardized values from the Clinical and Laboratory Standards Institute.

Isolation of R plasmids

Plasmid DNA will be isolated from *E.coli* strain(s) showing antibiotic resistance by alkaline lysis (Birnboim and Doly 1979) using commercially available Zyppy™ Plasmid Miniprep Kit from Zymo Research.

Plasmid profile analysis

Isolated plasmids will be characterized on the basis of their resistance specificity, molecular weights (electrophoresis on 1% agarose gel), restriction endonuclease (EcoRI) digest fragmentation patterns, and conjugative nature (bacterial conjugation with K12 plasmid-free *E.coli*). These data will be used to compare the isolates with *E.coli* R plasmids reported in the literature.

4. Expected timeline

Dates	Expected progress
March 3 rd -March 9 th	Preparation of media and requisition of supplies
March 10 th -March 16 th	Collection of samples from volunteer; identification and inoculation of <i>E.coli</i> .
March 17 th -March 28 th	Antibiotic sensitivity tests. Data collection
April 7 th -April 18 th	Plasmid isolation. Plasmid profile analysis. Data collection.
April 19 th -April 30 th	Final data analysis.

References

- Anderson, R.M. 1999. The pandemic of antibiotic resistance. *Nat. Med.* 5, 147-149.
- Barbosa, T.M., Levy, S. B. 2000. The impact of antibiotic use on resistance development and persistence. *Drug Resist. Updat.* 3, 303-311.
- Bimboim, H. C., & Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic acids research*, 7(6), 1513-1523.

WHO (World Health Organization), 2001. Global Strategy for Containment of Antimicrobial Resistance. 11-17.

5. Detailed Budget

Items	Quantities	Costs	Notes
VWR Petri Dish, 100x15 mm, slippable	1 Case of 500	\$170.24	Petri dishes available in the department are used for teaching purposes. It likely will be difficult to coordinate between large number of plates needed for both this study and for teaching labs.
Ampicillin (10ug) antibiotic disk. Fischer Technical No.:B31264	100 discs	\$34	Material for antibiotic sensitivity testing
Penicillin G (10 units) antibiotic disk Fischer Technical No.:B31321	100 discs	\$34	Material for antibiotic sensitivity testing
Tetracycline (30ug) antibiotic disk Fischer Technical No.:B31344	100 discs	\$34	Material for antibiotic sensitivity testing
Streptomycin (10ug) antibiotic disk Fischer Technical No.:B31328	100 discs	\$34	Material for antibiotic sensitivity testing
Ciprofloxacin (5ug) antibiotic disk BBL Microbiology (231657)	100 discs	\$49.64	Material for antibiotic sensitivity testing
Sulfamethoxazole antibiotic disk BBL Microbiology (231536)	100 discs	\$49.64	Material for antibiotic sensitivity testing
Zyppy™ Miniprep kit #D4019	100 preps	\$100	Material for plasmid isolation and profile analysis
	Total cost:	\$505.52	